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14. ABSTRACT

Parkinson's disease is caused, in part, by over expression and misfolding of a protein named alpha synuclein. The experiments in this year of the application were aimed at establishing a bilateral nonhuman primate model of Parkinson's disease focusing on replicating the over-expression and misfolding of alpha synuclein. Ten aged monkeys comprised this study. Following preoperative baseline testing, five monkeys received bilateral intranigral injections of an AAV vector encoding for human alpha synuclein. The remaining monkeys received identical injections of the reporter gene green fluorescent protein. For 16 weeks, monkeys received repeated beta-CIT SPECT scans to monitor dopaminergic function, clinical rating scale score, hand reach scores, and general activity measurements. None of these measures revealed differences between control and alpha synuclein treated monkeys. These animals have recently been sacrificed and are being evaluated for correct neuroanatomical placement and reasonable transgene expression in order to determine whether biological or technical issues resulted in the failure to create this model.

15. SUBJECT TERMS
Alpha synuclein overexpression may not provide a good nonhuman primate model for Parkinson's disease

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Introduction:

The studies performed this year were dominated by a study examining whether bilateral gene delivery of alpha synuclein to the substantia would induce a model of Parkinson's disease. The description of the study as performed and the results are described below. These animals underwent Quarantine, many months of preoperative testing, surgery, as well as post-operative testing.

Body:

Study Animals

10 laboratory-bred adult female rhesus macaques were used. The animals were housed at the UIC Biological Resources Laboratory. The primates weighted 4.4-8.0 kg and ranged in age from 21-24 years. All animals were singly housed with mesh-screen contact pairs to minimize the potential for injury for the duration of the study. The facility has a 12 hr on, 12 hr off lighting schedule with food and water available ad libitum.

Experimental Design

The goal of this study was to determine the effect of sustained bilateral expression of AAV6-alpha synuclein in the substantia nigra of rhesus macaques following 4 intracranial injections of the viral vectors. There were two groups of 5 animals with assignment to group based on baseline fine motor skills testing. Five animals were assigned to receive AAV6-alpha synuclein while a second group (the control group) received AAV6-GFP vectors.

Blinded Group Assignment:	
AAV6-GFP (Control)	AAV6-alpha synuclein
RH 7691	RH 7687
RH 7692	RH 7688
RH 7694	RH 7793
RH 7794	RH 7796
RH 7798	RH 7797

Intracranial Injection Procedure

The animals were anesthetized with Ketamine HCl (10 mg/kg), Domitor (2.55 mg/kg), intubated and placed in the stereotaxic frame for T1 MRI scans in a 3 Tesla unit machine. The coordinates for the target areas in the nigra were determined individually for each animal based on the MRI measurements. Animals were transported from the MRI unit directly to surgery in the stereotaxic frame. Animal were maintained on Isoflurane (1-3%) throughout the surgical procedure. In both experimental groups the animals received 2 bilateral injections in the substantia nigra. The infusion of the AAV vectors was performed with an infusion pump attached to the stereotaxic micromanipulator. The rate of infusion was 1.0 µl/min with a dose volume of 8 µl per

injection site. Under sterile conditions, a coronal incision was made over the scalp. Entry points were identified according to their distance from the MRI-calculated zero mark where a square entry hole was drilled for each site. The exposure of the superior sagittal sinus served as the midline zero. The dura was opened and measurements of the cortical surface were recorded. The same 50 µl Hamilton syringe with a 30 gauge blunt tip needle was used for each injection site in order. After each injection was completed, the needle was left in place for additional 5 minutes to allow the infusate to diffuse off the needle tip before slowly retracting the syringe. Identical infusion procedures were used for both experimental and control animals. Following the injections, the burr holes were filled with Gelfoam and the surgical site was closed in anatomical layers. Animal received the appropriate post-surgical care as deemed by the clinical veterinarian.

SPECT Scan Imaging

Most animals received SPECT scanning (Single Photon Emission Computed Tomography) pre-operatively and 2, 4, 8, 12 and 16 week intervals post-operatively using B-CIT (Dopascan), a dopamine receptor ligand radiolabeled with Iodine 123. A dopamine receptor specific ligand binds to the receptor site, allowing the percentage uptake and dopamine neuronal function to be quantified.

Animals were anesthetized with Ketamine HCl (10mg/kg), a catheter was inserted in either the saphenous or cephalic vein, induced with Propofol (1% emulsion), intubated and maintained with Isoflurane (1-3%) throughout the duration of the scan. Injections of B-CIT I^{123} were administered approximately 18 hours prior to the scan time per recommendation of the manufacturer. Animals were anesthetized with Ketamine HCl (10mg/kg) and a catheter was inserted in either the saphenous or cephalic vein. Injections of at least 1.0 mCi of radioactivity were required in order to obtain optimal images; volume of the injection was dependent upon activity. Scan time duration was approximately 1 hour per animal per scan, with 3 repetitions per scan assembled for image reconstruction. T1 MRI scans taken the day of surgery were also embedded and used for image reconstruction and analysis. Analysis was performed by Molecular Neuroimaging, Inc., New Haven, Connecticut.

The Table below shows the B-CIT scan results for animals injected with **AAV6-Alpha Synuclein** (N=5) from Baseline to 16 weeks post surgery.

Injection Date	Scan Date	Radio pharm aceuti cal	Dose Injected (mCi)	Mean counts in striatal V.O.I.	Mean counts in occipital V.O.I.	Normalized occipital counts	Age (years)	Animal Number	Time Point	Ratio to occipital (mean of all frames)
4/1/2009	4/2/2009	b-CIT	1.83	12662	747	408	22	RH 7687	Baseline	**17.08**
5/28/2009	5/29/2009	b-CIT	1.95	12307	844	433	22	RH 7687	4 Weeks	**15.36**
6/24/2009	6/25/2009	b-CIT	1.83	11998	779	426	22	RH 7687	8 Weeks	**15.49**
7/22/2009	7/23/2009	b-CIT	1.67	13390	1072	642	22	RH 7687	12 Weeks	**12.71**
8/19/2009	8/20/2009	b-CIT	1.46	12441	1045	715	22	RH 7687	16 Weeks	**11.95**
3/26/2009	3/27 /2009	b-CIT	2.68	14489	718	268	22	RH 7688	Baseline	**20.23**
5/28/2009	5/29 /2009	b-CIT	1.69	13326	718	425	22	RH 7688	4 Weeks	**18.77**

6/24/2009	6/25 /2009	b-CIT	1.49	14042	667	447	22	RH 7688	8 Weeks	**21.1**
8/5/2009	8/6/2009	b-CIT	1.41	13390	1072	760	22	RH7688	14 Weeks	**17.73**
8/19/2009	8/20 /2009	b-CIT	1.44	14174	865	601	22	RH7688	16 Weeks	**16.66**
3/26/2009	3/27/2009	b-CIT	2.76	14938	671	243	22	RH 7793	Baseline	**22.7**
5/28/2009	5/29/2009	b-CIT	1.77	15099	1239	700	22	RH 7793	4 Weeks	**12.22**
6/24/2009	6/25/2009	b-CIT	3	14866	766	255	22	RH 7793	8 Weeks	**19.46**
7/22/2009	7/23/2009	b-CIT	3.09	14474	996	322	22	RH 7793	12 Weeks	**14.58**
8/19/2009	8/20/2009	b-CIT	1.13	13775	645	571	22	RH 7793	16 Weeks	**21.93**
4/1/2009	4/2/2009	b-CIT	2.42	13490	657	272	22	RH 7796	Baseline	**21.51**
5/26/2009	5/27 /2009	b-CIT	1.75	12529	996	569	22	RH 7796	2 Weeks	**12.58**
6/10/2009	6/11 /2009	b-CIT	1.23	11507	932	758	22	RH 7796	4 Weeks	**12.33**
7/8/2009	7/9/2009	b-CIT	1.88	12410	961	511	22	RH 7796	8 Weeks	**12.92**

Injection Date	Scan Date	Radio pharm aceuti cal	Dose Injected (mCi)	Mean counts in striatal V.O.I.	Mean counts in occipital V.O.I.	Normalized occipital counts	Age (years)	Animal Number	Time Point (Interval)	Ratio to occipital (mean of all frames)
8/5/2009	8/6/2009	b-CIT	1.78	13731	770	433	22	RH7796	12 Weeks	**18.09**
4/1/2009	4/2/2009	b-CIT	2.18	13065	1288	591	22	RH 7797	Baseline	**10.15**
5/26/2009	5/27/2009	b-CIT	2.17	12612	1627	750	22	RH 7797	2 Weeks	**8.6**
6/10/2009	6/11/2009	b-CIT	1.25	12613	2350	1880	22	RH 7797	4 Weeks	**5.39**
7/8/2009	7/9/2009	b-CIT	1.65	11806	1480	897	22	RH 7797	8 Weeks	**8.08**
8/5/2009	8/6/2009	b-CIT	1.94	11504	1193	615	22	RH7797	12 Weeks	**9.66**

The Table below shows the B-CIT scan results for animals injected with **AAV6-GFP** (Control) (N=5) from Baseline to 16 Weeks post surgery.

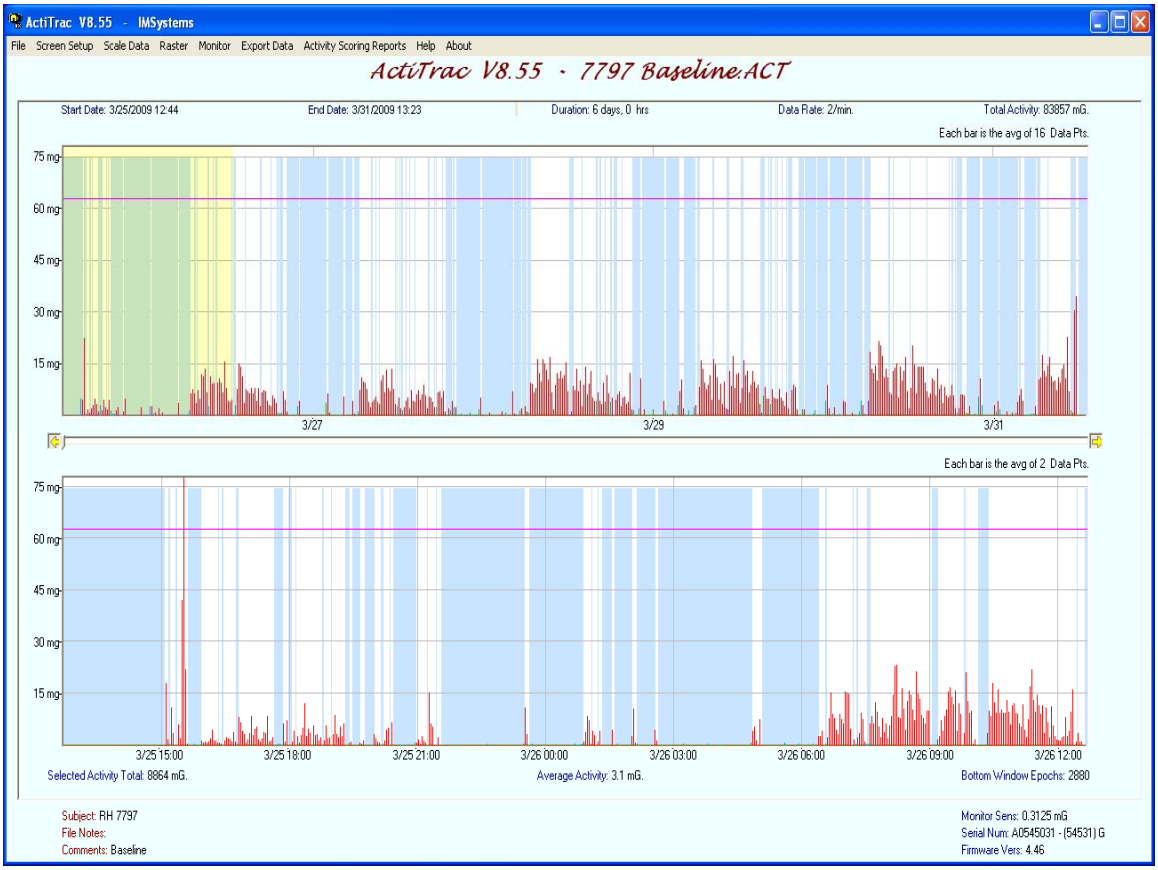
Injection Date	Scan Date	Radio pharm aceuti cal	Dose Injected (mCi)	Mean counts in striatal V.O.I.	Mean counts in occipital V.O.I.	Normalized occipital counts	Age (years)	Animal Number	Time Point (Interval)	Ratio to occipital (mean of all frames)
3/26/2009	3/27/2009	β-CIT	1.79	10086	568	318	22	RH 7691	Baseline	**17.91**
5/28/2009	5/29/2009	β-CIT	2.08	10049	578	278	22	RH 7691	4 Weeks	**17.42**
6/24/2009	6/25/2009	β-CIT	1.26	10725	546	433	22	RH 7691	8 Weeks	**20.01**
7/22/2009	7/23/2009	β-CIT	1.5	14983	856	571	22	RH 7691	12 Weeks	**15.2**
8/19/2009	8/20/2009	β-CIT	1.32	N/A	N/A	0	22	RH 7691	16 wk post	**N/A**
4/1/2009	4/2/2009	β-CIT	2.79	13596	719	258	23	RH 7692	Baseline	**19.26**
5/28/2009	5/29 /2009	β-CIT	2.24	14157	1115	498	23	RH 7692	4 Weeks	**12.7**
6/24/2009	6/25 /2009	β-CIT	1.63	11907	611	375	23	RH 7692	8 Weeks	**19.72**
7/22/2009	7/23 /2009	β-CIT	1.46	13068	961	658	23	RH 7692	12 Weeks	**13.64**
8/19/2009	8/20 /2009	β-CIT	1.19	N/A	N/A	0	23	RH 7692	16 Weeks	**N/A**
4/1/2009	4/2/2009	β-CIT	3.21	13800	755	235	23	RH 7694	Baseline	**18.29**
5/28/2009	5/29/2009	β-CIT	2.29	13099	846	369	23	RH 7694	4 Weeks	**15.5**
6/24/2009	6/25/2009	β-CIT	1.99	13223	679	341	23	RH 7694	8 Weeks	**19.73**
7/22/2009	7/23/2009	β-CIT	1.49	11408	833	559	23	RH 7694	12 Weeks	**14.01**

8/19/2009	8/20/2009	β-CIT	1.18	N/A	N/A	0	23	RH 7694	16 Weeks	**N/A**
3/26/2009	3/27 /2009	β-CIT	1.72	12735	475	276	24	RH 7794	Baseline	**26.9**
5/26/2009	5/27 /2009	β-CIT	2.14	12416	548	256	24	RH 7794	2 Weeks	**22.92**
6/10/2009	6/11 /2009	β-CIT	1.19	11612	578	486	24	RH 7794	4 Weeks	**20.58**
7/8/2009	7/9/2009	β-CIT	2.37	10569	542	229	24	RH 7794	8 Weeks	**19.55**
8/5/2009	8/6/2009	β-CIT	1.33	12836	725	545	24	RH7794	12 Weeks	**18.01**
4/1/2009	4/2/2009	β-CIT	2.04	13884	879	431	22	RH 7798	Baseline	**15.83**
5/26/2009	5/27/2009	β-CIT	2.25	12555	682	303	22	RH 7798	2 Weeks	**18.61**
6/10/2009	6/11/2009	β-CIT	1.72	11471	809	470	22	RH 7798	4 Weeks	**14.16**
7/8/2009	7/9/2009	β-CIT	2.19	11878	834	381	22	RH 7798	8 Weeks	**14.43**
8/5/2009	8/6/2009	β-CIT	1.51	10608	1338	886	22	RH7798	12 Weeks	**9.68**

No differences were observed between treatment groups.

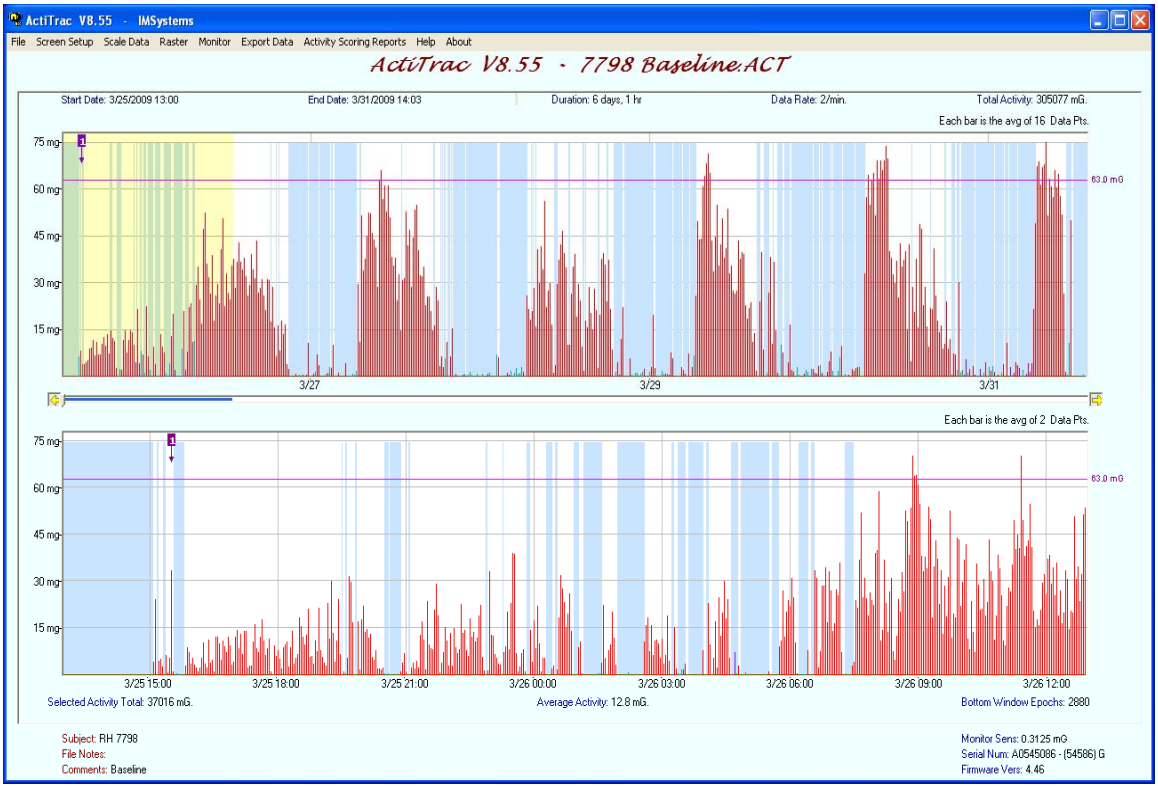
ActiTrac Physical Activity Monitors

Movement activity of each animal was obtained using ActiTrac motion sensor accelerometers. Accelerometers were used to track the difference in overall activity of all animals post surgical treatment. Animals were anesthetized with Ketamine HCl (10 mg/kg) and fitted with a properly sized jacket. Motion sensors were placed in a special pocket on the back of the jacket to prevent the animal from tampering with the device. Animals were fitted with jackets pre-operatively and allowed to acclimate for approximately 1 week; post-operatively, animals were monitored at 3, 7, 11 and 15 week (only 6 animals from surgery group 1) intervals. Data is recorded in milliG (mG) units, where G is the Earth's gravitational acceleration, 9.8 m/s^2. Sensors were programmed with the maximum sensitivity of 0.312 mG and an epoch of 30 seconds; light sensors were disabled. Data were collected for 5-7 days (24 hours/day) and specific time points were chosen for analysis at each interval for an accurate comparison between groups. Animals that received the nigral AAV6-alpha synuclein injections bilaterally were expected to display an overall decrease in average activity, while animals that received nigral AAV6-GFP injections bilaterally were expected to be behaviorally normal. Shown below are four example graphs of the activity data collected to be analyzed:

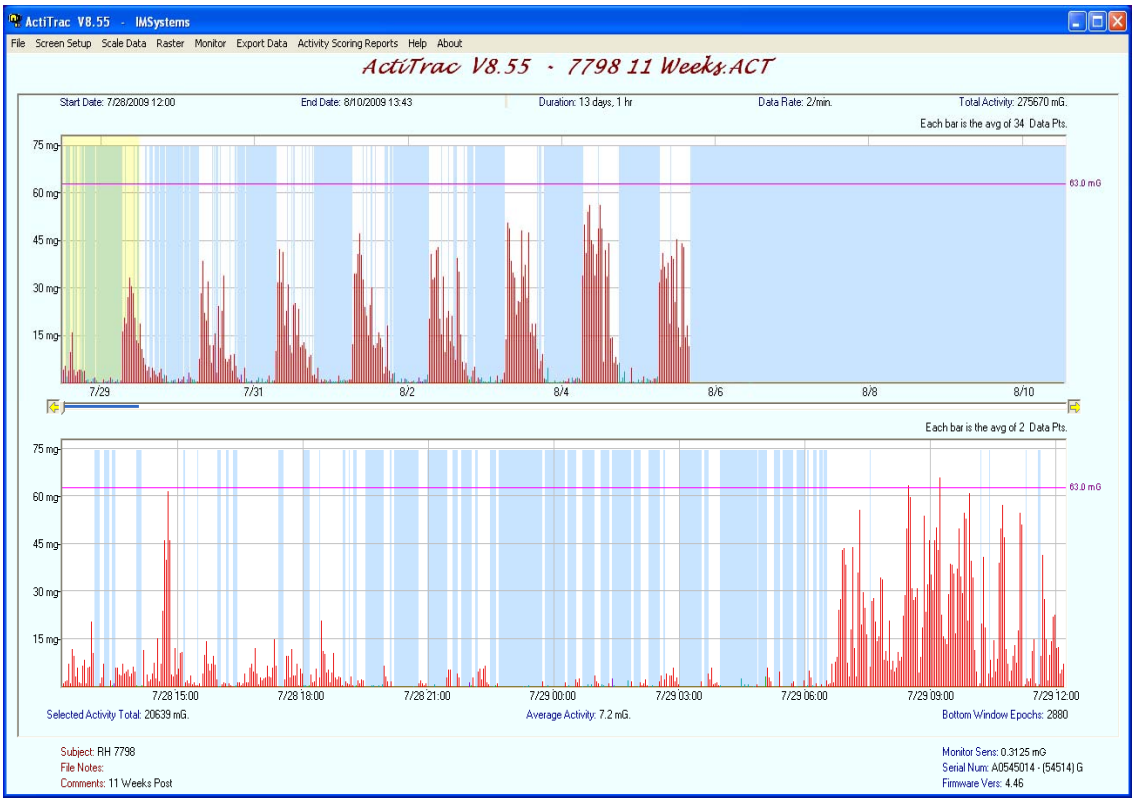


RH 7797 shown at Baseline data collection interval.

RH 7797 received **AAV6-Alpha Synuclein** injections. This activity data is shown at 12 weeks post surgery.



RH 7798 shown at Baseline data collection interval.

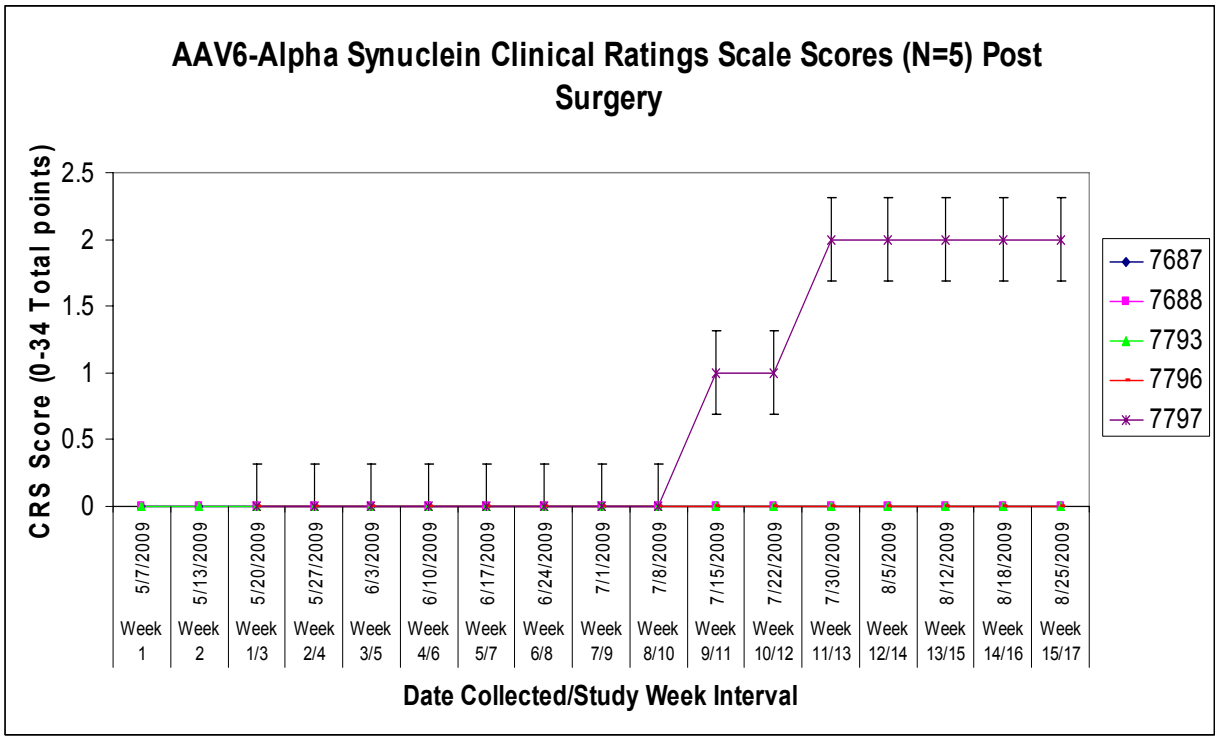


RH 7798 received **AAV6-GFP (Control)** injections. This activity data is shown at 11 weeks post surgery.

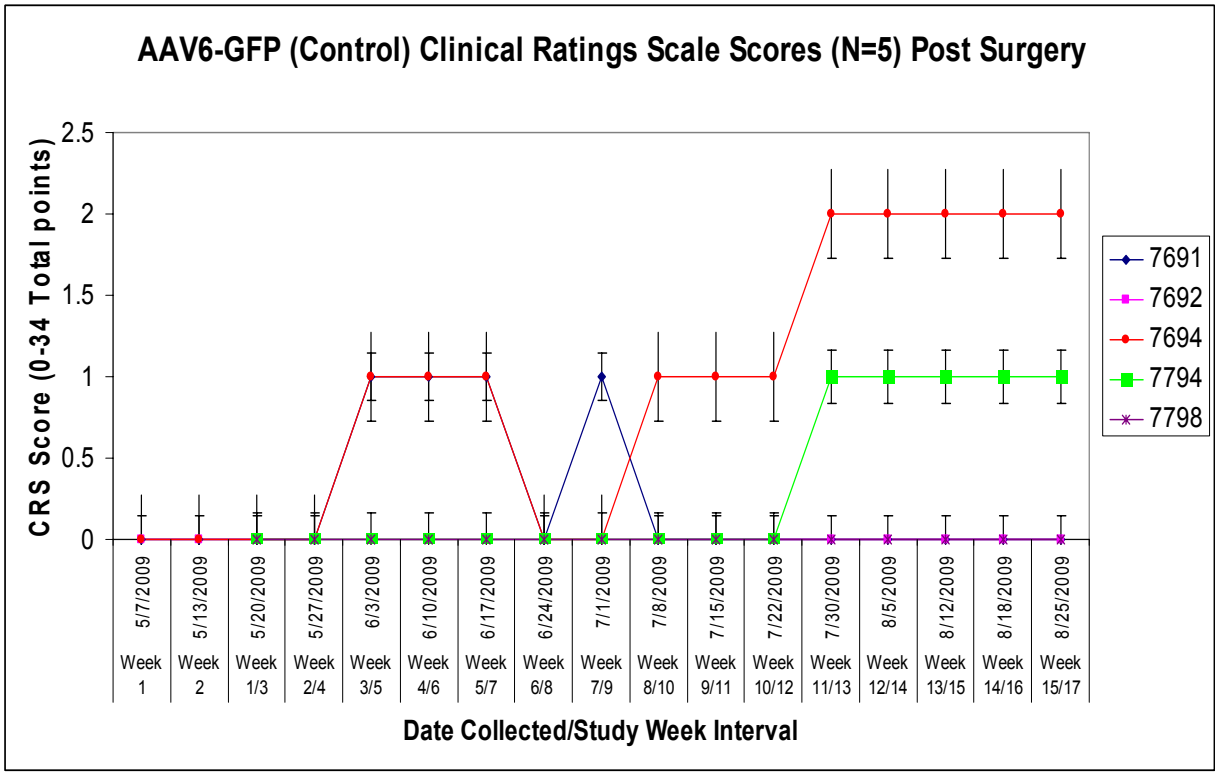
No differences in activity were demonstrated between the two groups.

Clinical Ratings Scale

The Clinical Ratings Scale was used to assess the clinical status of each animal under normal and post operative treatment conditions. A trained observer that was blinded to the treatment status of the animals performed all of the ratings. Animals were observed under the same environmental conditions in their home cage during each collection interval. The scale consists of observation rating categories for posture (0-3), gait (0-5), bradykinesia (0-5), balance (0-3), tremor (0-3) for each arm, gross motor skills (0-4) for each arm, defense reaction (0-2) and freezing (0-2) with a total of 34 points possible, 0 indicating a normal animal and 34 indicating a severely impaired animal. Occurrence of dyskinesias, psychological disorders and other abnormal behavior if observed were noted. CRS scores were evaluated 3 times pre-operatively and once weekly beginning 1 week post surgery up to Week 17 for surgery group 1 and Week 15 for surgery group 2. Baseline CRS scores were collected at 3 separate scoring intervals. All animals were clinically normal (CRS=0) prior to placement on study.



This graph shows the post-surgical CRS scores of the AAV6-Alpha Synuclein treated animals. All animals were clinically normal (CRS=0) at baseline.



This graph shows the post-surgical CRS scores of the AAV6-GFP (Control) treated animals. All animals were clinically normal (CRS=0) at baseline.

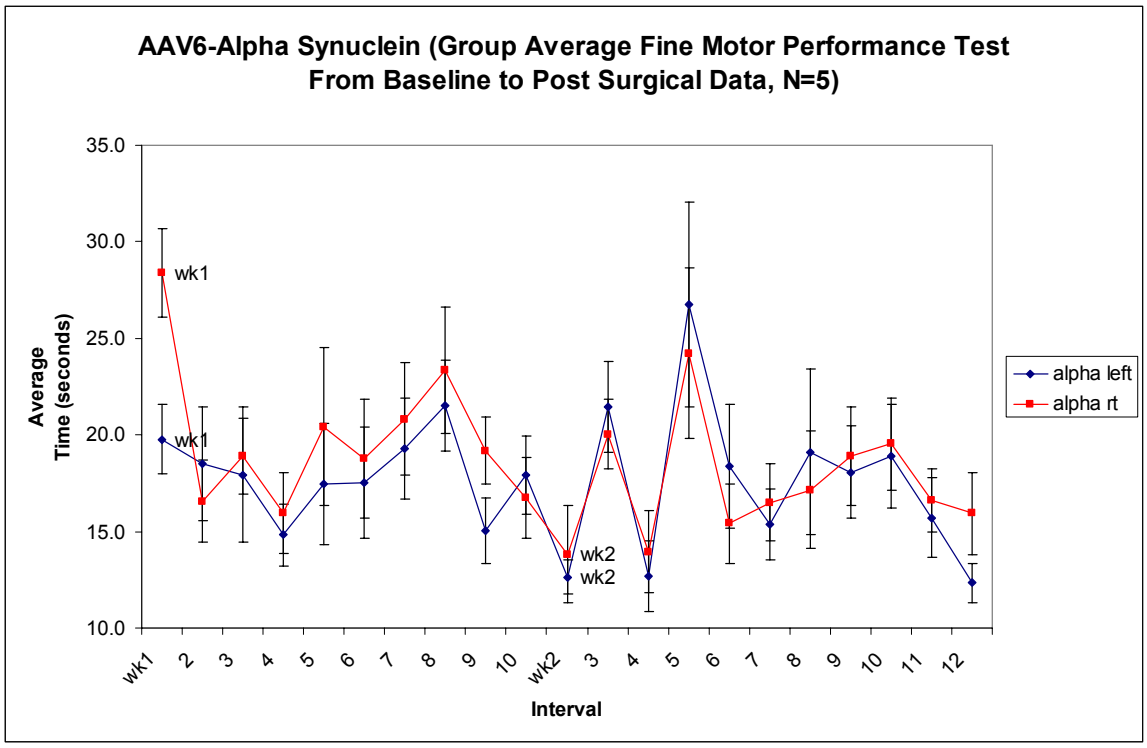
All animals remained clinically normal throughout the duration of the study, with the exception of 7794, 7797, 7691 and 7694. These animals scored ratings in the categories of bradykinesia, balance, defense reaction and tremor. No animal achieved a total CRS score greater than 2 points at any point post-surgery. There were no differences between groups.

Fine Motor Performance Testing

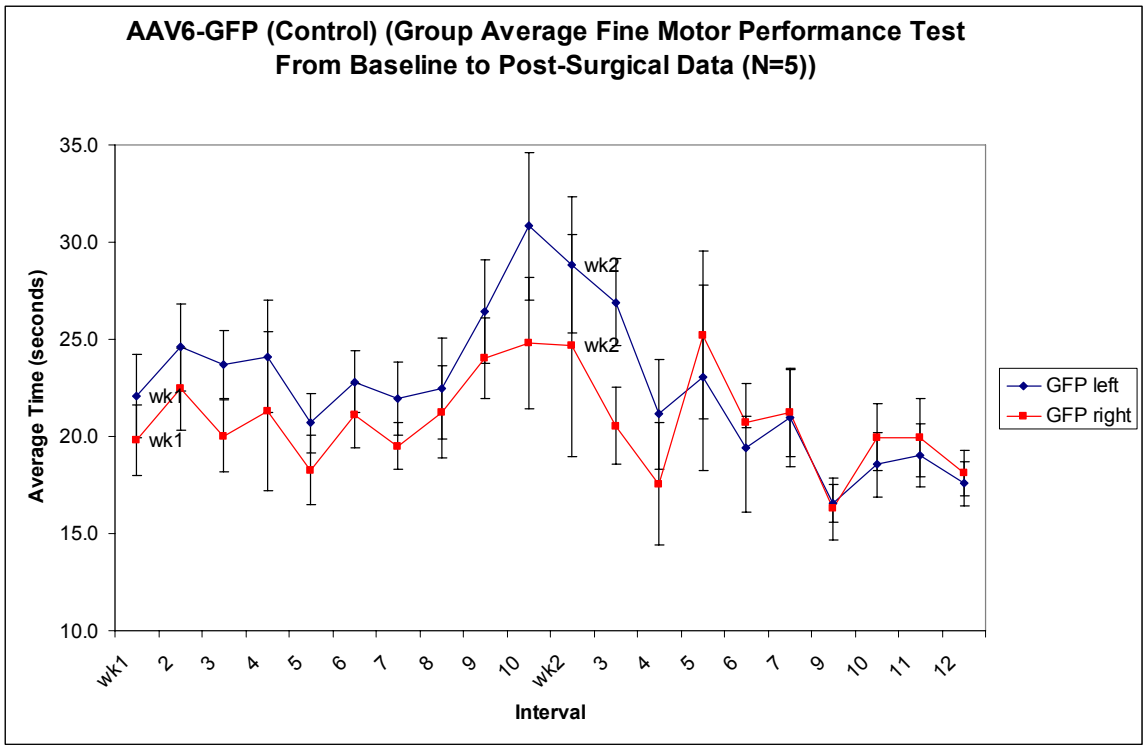
Fine Motor Performance Testing measures the animals' fine motor skills in the hand and fingers while also assessing their overall ability to use their upper limbs. All primates were trained on the fine motor performance test (hand reach test) prior to being placed on study. One trainer blinded to whether animals received AAV6-GFP or AAV6-alpha synuclein was present for all testing. Animals were introduced to a handheld transfer cage and brought from their home cage to a separate testing cage and room. An acclimation process (amount of time necessary varied per animal) was used to familiarize them with the cage, room and overall testing environment to minimize outside distractions. Animals were transferred to the testing cage and a small, divided plexiglass testing board was placed on the front of the cage. The divided sliding tray assembly consisted of 9x9 recessed wells on each side of the divider, corresponding to the left and right hand. Based on the design of the board and cage door, animals are only able to work with either the left or right hand at a time. Should the animal use the improper hand, the animal was corrected by removing the board and the food reward. Only six of

the nine totals wells were used for this particular test. Using only one side of the board at a time, small pieces of fruit, vegetable or treat were placed in the wells and the animal was timed on its pick up task performance. Time was recorded to the nearest tenth of a second, with recording beginning from the moment the animal places its finger into the first well until the moment it retrieves the last piece. Each animal was required to complete ten trials per collection period. If the animal stopped testing during the trial collection period, only those trials were collected and averaged into the overall score.

Surgery Group 1 began fine motor performance testing 3 weeks post surgery and surgery Group 2 began 2 weeks post surgery. For the group average graph analysis, baseline and post-surgical data were assembled; standard deviation and standard error of the mean were calculated for the AAV6-alpha synuclein (N=5) and AAV-GFP (N=5) group animals. Animals in either the test or control group did not always have data collected at the same intervals, which was due in part to other data being collected at the same time period. For example, radioactive quarantine for Iodine 123 used during the SPECT scans is a minimum of 5.5 days based on the half-life of I^{123}. For safety purposes, animals could not be transported to their testing room and cage until they had undergone the proper radioactive half-life. The data graphs below show the Fine Motor Performance Test data averages of the alpha-synuclein versus GFP treated groups, with no less than two animals being used for the average of any given data point.



This graph shows the AAV6-Alpha Synuclein injected group average Fine Motor Performance Test data from baseline to post-operative interval collection. Data point Wk 1 indicates the start of baseline data collection. Data point Wk 2 indicates the start of post-operative data collection. RED=right hand. BLUE=left hand.



This graph shows the AAV6-GFP injected group average Fine Motor Performance Test data from baseline to post-operative interval collection. Data point Wk 1 indicates the start of baseline data collection. Data point Wk 2 indicates the start of post-operative data collection. RED=right hand. BLUE=left hand.

There were no differences between groups.

Key research accomplishments: We were able to perform the study as proposed. We have yet to determine a parameter where the AAV6-alpha synuclein treated monkeys were impaired relative to controls. We are currently sacrificing the monkeys to determine gene expression and accuracy of injections. We are currently underway with out final experiment in which aged monkeys will receive SiRNA directed against alpha synuclein will be injected into the nigra in an attempt to diminish age-related alphasynuclein accumulation and its effect on dopamine expression.

Reportable Outcomes: None at this time.

Conclusions: Final conclusions will be made once the anatomical portion of this study is completed.

References: None

Appendices. None